animal

The effects of pathogen challenges on the performance of naïve and immune animals: the problem of prediction

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Predictive frameworks for performance under both physical and social stressors are available, but no general framework yet exists for predicting the performance of animals exposed to pathogens. The aim of this paper was to identify the key problems that would need to be solved to achieve this. Challenges of a range of hosts by a range of pathogens were reviewed to consider reductions in growth beyond those associated with reductions in voluntary food intake (VFI). Pair-feeding and marginal response studies identified the extent and mechanisms of how further reductions in growth occur beyond those caused by reduced VFI. Further reductions in growth depended on the pathogen, the host and the dose and were time dependent. In some instances the reduction in VFI fully explained the reduction in growth. Marginal response experiments showed increased maintenance requirements during exposure to pathogens, but these were different for specific amino acids. There were no clear effects on marginal efficiency. Innate immune functions, repair of damaged tissue and expression of acquired immunity caused significant but variable increases in protein (amino acid) requirements. More resistant genotypes had greater requirements for mounting immune responses. The partitioning of protein (amino acids) was found to be different during pathogen challenges. Prediction of the requirements and partitioning of amino acids between growth and immune functions appears to be a crucial problem to solve in order to predict performance during pathogen challenges of different kinds and doses. The problems of accounting for reductions in performance during pathogen challenges that are described here provide a useful starting point for future modelling and experimental solutions.

Introduction

Predicting the effects of physical (Black *et al.*, 1986; Wellock *et al.*, 2003a), social (Wellock *et al.*, 2003b) and infectious (Black *et al.*, 1999) stressors on performance is important for guiding future management, genetic selection and experimental strategies. No general model exists that predicts growth and performance of different host genotypes, when given access to different kinds of foods and challenged by pathogens. The aim of this paper was to characterise reductions in growth during exposure to different kinds and amounts of pathogen that were not caused by a reduction in voluntary food intake (Sandberg *et al.*, 2006). This allowed for the main consequences of pathogen challenges that would need addressing in a predictive framework of growth to be identified.

There is no general agreement on the overall problems that need solving in order to predict growth during pathogen challenges (Black *et al.*, 1999; Coop and Kyriazakis, 1999; Kyriazakis, 2003; Powanda and Beisel, 2003). A general comprehensive model can provide a clearer background for the design of future experiments, and help improve our current understanding of the interactions between pathogen challenge, the host and its nutrition. To achieve this, three kinds of problems were considered in this review. The extent of reductions in growth independent of those caused by reduced voluntary food intake was assessed using pair-feeding and marginal response experiments. Data that permitted further characterisation of changes in requirements during pathogen challenges were then considered. The final issue considered was the partitioning of scarce protein and energy between maintenance, growth and immune functions.

Sandberg *et al.* (2005a and b) described solutions to the partitioning of scarce resources in healthy animals. A solution which predicted the marginal response in protein retention, *PR* g/day, to ideal protein intake from the energy

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to protein ratio of the food (Kyriazakis and Emmans, 1992a and b) agreed well with literature data. This solution, combined with protein (Moughan, 2003) and energy (Emmans, 1994) systems, allows the prediction of actual growth as rates of *PR* and lipid retention, *LR* g/day. To predict growth during health, sub-clinical and clinical disease it is necessary to test for consistency with the components of the above framework, and identify any additional components, which apply during pathogen challenges.

Previous reviewers have considered one or several effects of pathogen challenges on voluntary food intake, resource requirements or partitioning in relation to the ability of an animal to cope with a challenge. Reviews have been from the perspectives of animal production (Van Houtert and Sykes, 1996; Coop and Kyriazakis, 1999, 2001; Koutsos and Klasing, 2001), human medicine (Powanda, 1977; Powanda and Beisel, 2003) and evolutionary ecology (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000). The overall problem considered in all of these fields was how pathogen challenges (kind, dose and virulence) affect the level of disease and rate of growth of an animal, when given a certain kind of food. The approach taken here is different, in that it considers the consequences of pathogen challenges that need including in a predictive framework. The requirements and partitioning of both protein (amino acids) and energy were considered for different pathogens and hosts. The critical analysis of literature evidence combines to provide a description of the overall problem of reduced performance during pathogen challenges, and may provide a starting point for predictive and experimental solutions.

Accounting for reductions in growth during pathogen challenges

The following conceptual equation (e.g. Parks, 1982) provides a starting point for accounting for reductions in rates of growth, d*W*/d*t*, during different pathogen challenges, which is the problem considered here:

$$dW/dt = e_w \cdot (VFI - m.W) \qquad [1]$$

where e_w is the net efficiency of using a food for live-weight gain, *VFI* is the voluntary food intake and *m* represents the multiplier of live weight, *W*, to determine requirements for maintenance. Reductions in *VFI* (Sandberg *et al.*, 2006) or efficiency of resource use (Sykes, 2000), or, increased maintenance requirements (Black *et al.*, 1999; Houdijk *et al.*, 2001) could lead to reductions of d*W*/d*t*. Reductions in growth could also occur due to a combination of these factors. Further 'marginal response' relationships have been proposed that relate to specific resources such as protein or energy intakes to *PR* and *LR* e.g. Kyriazakis and Emmans (1992a and b) and Black *et al.* (1986). The problem was to identify which components of these relationships may be affected during pathogen challenges.

To assess the effect of pathogen challenges on components other than *VFI* in equation [1] (and equivalent parameters for other marginal responses) data from pair feeding and marginal response experiments were first considered. The aim was to identify the extent, and the mechanism(s), by which growth was reduced during pathogen challenges. When considering marginal response experiments it seemed necessary to consider also the effects of pathogen challenges on resource digestibility and protein quality (biological value). Other kinds of evidence for quantifying the causes of reductions in growth beyond those caused by a reduction in food intake such as repair of damage, cost of mounting an immune response and fever were then considered.

The characteristics of reductions in growth during pathogen challenges

Responses in growth during pair-feeding

To compare rates of growth of an uninfected and a challenged animal a pair-feeding method can be used to eliminate differences in growth due to differences in *VFI*. One of the main effects of exposure to pathogens is a reduction in *VFI* (pathogen induced anorexia, Kyriazakis *et al.* (1998)). The healthy control is given the same amount of food as the challenged animal, at a time, but for practical reasons often with at least one day's delay. In principle, their rates of growth can then be compared to quantify reductions in growth that are not associated with reduced *VFI* i.e. changes in digestibility or biological value, requirements or partitioning.

Parasitic challenges. Experiments with abomasal parasites such as *Teladorsagia* (*Ostertagia*) *circumcincta* and *Ostertagia ostertagi* have shown that challenged animals grew slower than their pair fed controls (Sykes and Coop, 1977; Fox *et al.*, 1989). The average live weight gains of sheep challenged with *Teladorsagia circumcincta* were 0.79 of their pair fed controls (Sykes and Coop, 1977). Nitrogen balances performed at weeks 2 to 3, 7 to 8 and 12 to 13 post infection demonstrated time dependent effects on the nitrogen balance: live weight gains for the challenged sheep for weeks 2 to 8 were 0.66 of the pair-fed controls. Reductions in growth in naïve hosts are related to the time course of an infection, and may reflect the acquisition and expression of immunity (Coop and Kyriazakis, 1999).

Mansour *et al.* (1991 and 1992) challenged calves with either a single, or a trickle dose of *Ostertagia ostertagi* and found no difference in average growth rate between challenged calves and their pair-fed controls. The observation may have been due to the size of the challenge doses: the single dose was 609 larvae per kg body weight (BW) and the trickle dose was seven larvae per kg BW per day. Fox *et al.* (1989) using the same type of parasite, but a larger trickle dose (98 larvae per kg BW per day) found differences in growth rate between challenged calves and

pair-fed controls. The effects of abomasal parasites on growth, beyond effects on *VFI*, depended on both the level of challenge and the stage of infection.

The immunological state of an animal, i.e. whether it is naïve to a pathogen or whether it has acquired immunity, needs to be accounted for when comparing rates of growth of challenged and healthy animals. Takhar and Farrell (1979b) found no differences in growth rate between immune chicks (data indicated increased *VFI*) and healthy pair-fed controls when re-challenged with *Eimeria acervulina* or *Eimeria tenella*. There were, however, clear differences in growth between pair-fed controls and immunologically naïve chicks. Immune animals may cope with larger challenge doses compared with immunologically naïve animals before effects on growth occur.

Kimambo *et al.* (1988) challenged sheep with the intestinal parasite *Trichostrongylus colubriformis* and found the average d*W*/d*t* of the challenged sheep to be 0.11 and 0.83 of the pair-fed controls between weeks 6 to 13 and 13 to 20, respectively. As indicated by faecal egg counts and blood eosinophil counts the sheep were starting to express immunity somewhere during weeks 6 to 13. During weeks 13 to 20 the sheep were fully immune with faecal egg counts reduced to zero, but as this was a continuous, trickle challenge) blood eosinophil remained elevated. The reduction in growth during weeks 13 to 20 suggested a cost of expressing immunity. The findings of MacRae *et al.* (1979) are in quantitative agreement, and those of Symons and Jones (1975), Poppi *et al.* (1986) and Datta *et al.* (1998) agree qualitatively.

Pair-feeding experiments with parasites other than those affecting the stomach and small intestine have also shown reductions in growth beyond those caused by reductions in food intake. The liver parasite *Fasciola hepatica* given at a low dose (three *metacercariae* per host) did not cause any significant differences in the rate of growth or body composition between parasitised sheep and their pair-fed controls (Sykes *et al.*, 1980). At higher doses (eight and 14 *metacercariae*), the challenge did have effects on growth compared with pair-fed controls. These findings are in support of those for abomasal parasites where reductions in growth, when compared with pair-fed controls, depended on the level of challenge.

Akinbamijo *et al.* (1997) found that calves of two breeds (N'Dama and Gobra zebu bulls) challenged with a blood borne parasite (*Trypanosoma congolense*) grew slower than their pair-fed controls, but the two breeds were not affected to the same extent. Abbott *et al.* (1985) also found effects of genotype (Finn Dorset *v.* Scottish Blackface sheep) and nutrition (high or low protein) on differences in growth rate between sheep challenged with *Haemonchus contortus* and their pair-fed controls. In the case of the effect of nutrition, the lower reduction in growth seen for the higher protein diet would have been associated with the extent of damage. The effect of host genotype is more difficult to explain. It may be the outcome of greater genetic resistance, an outcome of damage affecting a genotype to a lesser extent than others, or the outcome of genotypes used (growth potential and resistance) and the level of nutrition. A framework of growth during pathogen challenges would need to distinguish between these factors causing reductions in growth.

Bacterial challenges. Experiments with intravenously administered bacteria (Ruot *et al.*, 2000; Papet *et al.*, 2002), lipopolysaccharide, *LPS,* (Steiger *et al.*, 1999), and local (nasal or oral) bacterial (Wannemacher *et al.*, 1971; Powanda *et al.*, 1972; Wannemacher *et al.*, 1974), or, bacterial antigen challenges (Klasing *et al.*, 1987; Arnold *et al.*, 1989) have shown reductions in growth that were not accounted for by reduced *VFI*. Challenges with *LPS,* while not a 'true' bacterial challenge, stimulate a similar kind of response, but of smaller and shorter magnitude, to that of bacterial challenges. As for macro-parasites these effects were dose dependent; Hunter and Grimble (1997) and Raina *et al.* (2000) failed to demonstrate any reductions in growth that were not accounted for by reduced *VFI* in *LPS* challenged rats.

Klasing *et al.* (1987) challenged growing chicks with non-pathogenic antigens (sheep red blood cells or sephadex), bacterial antigens (*Escherichia coli* or *Salmonella typhimurium LPS*) or heat killed *Staphylococcus aureus.* Pair feeding was done four times per day to account for rapid effects of bacterial antigens on *VFI*. The relative growth rates (challenged growth rate/control growth rate) of chicks challenged with non-pathogenic antigens (0.90) and pathogenic antigens (0.83) were different. This may suggest that a host may respond in relation to the kind of pathogen challenging it (for a review see Baxter and Hodgkin (2002)). The more virulent pathogen may have stimulated a greater immune response, rather than causing more damage to the host and through a larger resource requirement of the greater immune responses had greater reductions in growth (see below).

Viral challenges. A few experiments were identified that considered the effects of viral challenges on growth in pair-fed animals compared with parasitic and bacterial models. Zijlstra *et al.* (1997) found that pigs (starting weight ≈ 1.5 kg) infected with *rotavirus* grew slower than their pair-fed controls. The findings of Koyama *et al.* (1997) agree as rats challenged with *adenovirus* also grew slower than their pair-fed controls. Roberts and Almond (2003) found no difference between pigs challenged with *porcine respiratory reproductive syndrome* (*PRRS*) virus and *Mycoplasma hyopneumoniae* and their pair fed controls. This may have been due to the pathogen or dose used, or due to the nature in which the data was analysed (average growth rate calculated over several weeks). Several experiments have shown that reductions in growth beyond those caused by reduced *VFI* were variable over the time course of pathogen challenges.

The experiments considered here included a variety of pathogens and hosts. Naïve and immune animals

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responded differently, with little or no reductions in growth in immune animals. The extent of unaccounted reductions in growth in immunologically naïve animals during pathogen challenges were, as expected, both pathogen (kind and dose) and time dependent. The literature data suggested, however, that over a certain range of doses, reductions in *VFI* fully explained reductions in growth. Effects on growth, beyond reduced *VFI*, depended on both host genotype and nutrition. To assign mechanisms for these effects on growth, marginal responses are now considered.

Responses in growth to resource intake
Marginal response experiments determine the amount of food (or resource) that is required for an animal to maintain component weight; thereafter rates of growth increase along the margin until a maximum response is achieved. In equation [1] weight gain is made a function of *VFI*. It may be better to consider the response to a specific resource (either protein or energy), as it may allow for a better accounting of the effects of pathogen challenges. The relationship between protein retention, *PR* g/day, and ideal protein intake, *IP* kg/day is a typical example of a marginal response. *IP* is the product of *VFI*, the crude protein content of the food *CPC* (kg/kg), its true ileal digestibility, d_{CP} and the biological value of the protein (see below), v:

$$PR = e_p.((VFI.CPC.d_{CP}.v) - MP) \qquad [2]$$

where e_p is the marginal response of *PR* to *IP*, above maintenance, *MP* kg/day, and the relationship applies when $PR < PR_{max}$ g/day, the animals' maximum genetic potential for *PR*. Both energy supplies in relation to protein intakes (reviewed by Sandberg *et al.* (2005b)) and stressors (Wellock *et al.*, 2003b) may prevent the attainment of PR_{max}. To try to identify how reductions in growth occur during pathogen challenges, food digestibility, biological value and marginal responses (including PR_{max}, *MP* and e_p) are now considered in turn.

The digestibility of food during pathogen challenges. A pathogen challenge may affect the ability of a host to break down and absorb organic matter causing a reduction in available protein and energy (Turk, 1972). The literature is not consistent, however, on whether there are effects on true or apparent digestibility (Sykes and Greer, 2003). For this purpose it is important to consider the main site of infection of a particular pathogen. A pathogen that causes damage to the gastro-intestinal tract (GIT) could affect the ability of the host to digest organic matter components of a food. On the other hand, pathogens that do not affect the GIT, would not be expected to affect either digestion or absorption (Klasing and Barnes, 1988).

The apparent digestibility of protein was reduced during pathogen challenges with parasitic worms of the stomach, small and large intestine in pigs (Hale, 1986). The reduction in apparent digestibility was smaller for pathogens that affected mainly the stomach, compared with parasites of the small and large intestine (Hale, 1986). The additional nitrogen excreted in the faeces, however, may have been associated with nitrogen leakage due to tissue damage and other endogenous nitrogen containing secretions e.g. mucin or plasma, and not due to a reduction of organic matter digestibility. Pathogen challenges affecting the small intestine, or parts further down the GIT, may result in damaged (or leaked) tissue not being digested and reabsorbed, while endogenous nitrogen leaving the stomach may be fully or partly re-absorbed in the small intestine. The observation that parasites of the small intestine (*Trichostrongylus colubriformis*: Kimambo *et al.*, 1988) cause effects on growth beyond those associated with reduced *VFI* when compared with parasites of the stomach (*Teladorsagia circumcincta:* Sykes and Coop, 1977) is in support of this.

The true digestibility of organic matter during pathogen challenges has also been estimated. Symons and Jones (1970) estimated the true digestibility of protein using radioactively labelled protein in monogastrics (mouse and rat) and ruminants (sheep). They found no effect of large single doses with either one or three different gastro-intestinal nematodes (*Nematospiroides dubius*, *Nippostrongylus brasiliensis* and *Trichostrongylus colubriformis*) on the true digestibility of protein. Poppi *et al.* (1986) using duodenal and ileal cannulas in sheep continuously infected with a sub-clinical trickle dose of *Trichostrongylus colubriformis* found a reduction in apparent digestibility, but no effect on the true digestibility of nitrogen. Sykes and Greer (2003) considering further evidence for sheep are in agreement.

At low to moderate level challenges of the GIT, it appears that the true digestibility of food resources is not affected. It is likely that any effects on the ability of the host to digest organic matter will depend on the extent of damage caused by the pathogen. Damage incurred by a host increases with increasing levels (doses) of a challenge (Symons *et al.*, 1981) and the virulence (Turk, 1972) of a pathogen. It may be necessary to identify the doses and virulence at which true digestibility and absorption could be affected. This would allow the important distinction to be made between pathogen challenges that cause either only damage, or, damage and reductions in true organic matter digestibility.

The ideal amino acid composition of food protein during pathogen challenges. Once reductions in *VFI* and any effects on the digestibility of food resources have been taken into account, the biological value is the final measure of protein (amino acid) supply. The biological value (denoted by v in equation [2]) is calculated from the ratio of the first limiting amino acid (*AA*) in the food protein to that in a reference protein e.g. pigs whole body protein. The aim of this section was to determine how immune proteins differed in their amino acid contents, *AAC* g *AA* per kg CP, to that of a 'reference protein' used for healthy animals, as v is an important measure of protein supply. The ratio of essential to non-essential *AAs*,

becomes particularly relevant for low protein foods (Deschepper and de Groote, 1995), as the total amount of non-essential AAs may become limiting.

Animals may produce significant amounts of certain nitrogenous compounds such as cytokines, antibodies, acute phase proteins or specific immune cells during pathogen challenges. Beisel (1977) and Wannemacher (1977), amongst others, have proposed that such immune responses lead to specific requirements for certain amino acids. Immune proteins and cells have been found to contain high proportions of certain AAs (Reeds et al., 1994). Immune cells may also have high requirements for certain essential e.g. sulphur AA (Grimble and Grimble, 1998) and non-essential e.g. glutamine and arginine (Newsholme, 2001; Field et al., 2002) AAs. Table 1 summarises the AAC of acute phase proteins (Reeds et al., 1994), other immune proteins (Houdijk and Athanasiadou, 2003), colostrum and milk (Csapo'-Kiss et al., 1995) and a reference protein (whole body protein of pigs; Kyriazakis et al. (1993)).

The comparison of colostrum and milk is included as colostrum contains a large proportion of maternal antibodies. There are some marked differences between the different kinds of proteins in terms of their AAC. The proteins considered in Table 1 included proteins part of the innate immune responses (acute phase proteins) and proteins normally associated with expression of acquired immunity (immunoglobulins).

The AAC of acute phase proteins, APPs, was calculated as the average of six acute phase proteins (C-reactive protein, fibrinogen, alpha-1-glycoprotein, alpha-1-antitrypsin, haptoglobin and amyloid A) and compared with the reference protein. Large differences were noted (AAC of APPs v. AAC reference protein) for phenylalanine, tyrosine, tryptophan and threonine. The largest differences between the average of the two immunoglobulins and the reference protein were tryptophan, valine, cysteine and threonine. Serine, a non-essential AA, was present at greater concentrations in both kinds of immune proteins, and especially in immunoglobulins, and MacRae (1993) has discussed its potential importance during pathogen challenges.

There were very large differences for the amino acid contents of immune proteins to that taken to be the normal reference amino acid content. The important issue, however, is whether the total requirement for 'ideal protein' for potential growth, PR_{max}/e_p, and that for making immune proteins, IMP/e_p g/day, and their amino contents lead to a continuously changing reference protein in challenged animals. It is assumed that PR_{max} is the animal's genetic maximum for protein retention and that IMP is the amount of immune protein produced at a particular point in time. It is also assumed that e_p is the same for both challenged and healthy animals. During health, v is taken as a constant for a given kind of food and reference protein. As the value of IMP changes over the time course of an infection, it may be necessary to calculate v by

Table 1 A summary of the amino acid composition of different proteins that are associated with the immune response, in relation to the reference protein that is normally used for calculating the biological value of food protein. The amino acid composition of colostrum (a source rich in immune proteins), milk and reference protein (whole body protein of pigs) are also shown for comparison

	Amino acid composition (g/kg protein)[†]												
	Pig protein	Milk	Colostrum	APP1[§]	APP2[§]	APP3[§]	APP4[§]	APP5[§]	APP6[§]	IgA	IgE	SheepMCP	Mucin
Phenylalanine	38	18	24	105	46	64	83	30	103	26	30	29	15
Tyrosine	26	9	22	50	56	74	27	70	67	24	43	29	13
Tryptophan	8	–	–	42	35	30	11	32	45	26	20	8	–
Leucine	74	11	59	91	62	101	124	82	29	120	87	90	31
Isoleucine	35	5	29	54	32	48	49	47	29	13	39	69	15
Valine	47	54	145	77	48	46	59	84	18	96	80	73	222
Lysine	71	28	99	71	77	75	92	92	33	39	57	53	18
Histidine	28	21	100	16	27	17	37	38	35	11	18	29	–
Metionine	18	1	6	16	32	11	28	16	22	6	7	33	–
Cysteine	10	2	8	13	15	18	6	24	0	39	28	24	85
Threonine	38	115	46	58	60	74	66	54	30	75	103	45	224
Arginine	67	6	25	36	84	52	23	28	116	32	39	57	16
Proline	71	52	39	44	48	34	41	44	34	75	67	49	83
Glycine	91	32	63	46	59	19	33	44	61	69	52	86	49
Serine	40	288	88	84	91	31	49	40	47	126	103	86	65
Alanine	65	21	58	31	29	36	43	54	106	56	46	69	42
ASX[‡]	86	19	46	82	113	102	106	113	128	75	82	57	56
GLX[‡]	137	318	145	112	119	173	136	115	87	94	93	90	64

[†] Amino acid composition of whole body pig protein from Kyriazakis et al. (1993), acute phase proteins from Reeds et al. (1994) and for IgA, IgE, sheep mast cell proteases (MCP) and mucin were taken from Houdijk and Athanasiadou (2003).
[‡] ASX – Asparagine + aspartate. GLX – Glutamine + glutamate.
[§] APP(X) represents six different acute phase proteins: (1) C-reactive protein, (2) fibrinogen, (3) alpha-1-glycoprotein, (4) alpha-1-antitrypsin, (5) haptoglobin and (6) amyloid A.

weighting the requirements and AAC of the protein requirements for growth and immune responses:

$$v = (((((PR_{max}/e_p).AAC_{growth})$$

$$+ (IMP/e_p).AAC_{im}))/(PR_{max} + IMP)/e_p))/AAC_{food} \quad [3]$$

where AAC_{growth} is the normal reference protein, AAC_{im} is the AAC of the immune proteins and the content of the food protein is AAC_{food}. The potential protein retention (PR_{max}) is used in equation [3] as it is necessary to estimate v from potential protein requirements before predicting the partitioning of ideal protein towards actual PR. As PR_{max} (due to host state) and IMP (due to stage of infection and level of challenge) change over time it would be necessary to perform the above calculation on a time step basis. Modelling simulations would provide a more robust test of whether the ideal protein concept needs replacing (or revising) during pathogen challenges by taking into account changes in requirements for individual amino acids for both growth and immune functions.

Effects of pathogen challenges on the upper limit for growth. It is reasonable to assume that a given animal in a given state has an upper limit for growth e.g. Emmans and Fisher (1986). In marginal response experiments, it is assumed that this limit is achieved when no further response is observed to additional levels of resource intake: the linear-plateau concept. During pathogen challenges it would appear that challenged animals have not achieved the same upper limit for growth as their healthy controls (Willis and Baker, 1981a and b; Williams *et al.*, 1997a, b and c; Webel *et al.*, 1998a). The authors' conclusion was that the 'potential upper limit' for growth was reduced.

Webel *et al.* (1998a) found a lower plateau (maximum) for PR for LPS challenged chicks than their healthy controls, while Willis and Baker (1981a and b) found that chicks challenged with *Eimeria acervulina* had a lower plateau for average daily body weight gain. The reduction in PR_{max} was estimated roughly to be 25% from Williams *et al.* (1997a, b and c) for pigs in a high immune stimulation environment, while Webel *et al.* (1998a and b) found a reduction of 11% for the response to lysine, but not for threonine or arginine in chicks. A plateau, however, may also arise due to insufficient amounts of energy in relation to protein, or due to exposure to a non-infectious stressor (Kyriazakis and Emmans, 1992b; Wellock *et al.*, 2003b).

Kyriazakis and Emmans (1992b) showed that a reduction in the marginal efficiency for growth produces a plateau response, and that the marginal efficiency depended on the energy to protein ratio of the food. Williams *et al.* (1997a, b and c) used foods with different energy to protein ratios, and the highest level of protein, which was the only level that yielded a plateau response, had an energy to protein ratio close to the critical value for a reduction in efficiency to occur (Sandberg *et al.*, 2005b). The findings of

Webel *et al.* (1998a and b) and Williams *et al.* (1997a, b and c) do not, as some authors have assumed e.g. Escobar *et al.* (2004), necessarily show that an animal's upper limit for growth is reduced during pathogen challenges.

A reduction in appetite (anorexia) could also result in the kind of responses that have been observed for marginal responses. The experiments of Willis and Baker (1981a and b), Williams *et al.* (1997a, b and c) and Webel *et al.* (1998a) were performed over a defined time, rather than weight range. Effects of pathogen challenges on growth are often transient, with an initial drop in growth rate, after which the animals recover as shown in the example from Hein (1968) in Figure 1.

The transient drop (2 to 3 days long) in growth rate shown in Figure 1 dictated the extent to which the healthy and challenged animals differed in size after they had overcome the infection (post infection). The extent of the difference depended on the dose of oocysts of *Eimeria acervulina*. As the challenged animals were smaller post infection they would also have a smaller upper limit for growth at a time, compared with healthy controls. In the marginal response experiments that were mentioned earlier, calculations of average growth rates included post-infection growth rates. This would bias the average growth rate of challenged animals downwards, but the difference would be detectable at only higher protein intakes.

Bown *et al.* (1991) found that sheep given abomasal infusions with nitrogen, when challenged with a gastro-intestinal parasite achieved the same level of nitrogen retention as their healthy controls; thus their data do not support a reduced upper limit for growth. It is difficult to design an experiment that would allow the two mechanisms (reduced potential versus reduced appetite) to be distinguished. A possible solution could be to combine the experimental methodologies of pair feeding and marginal
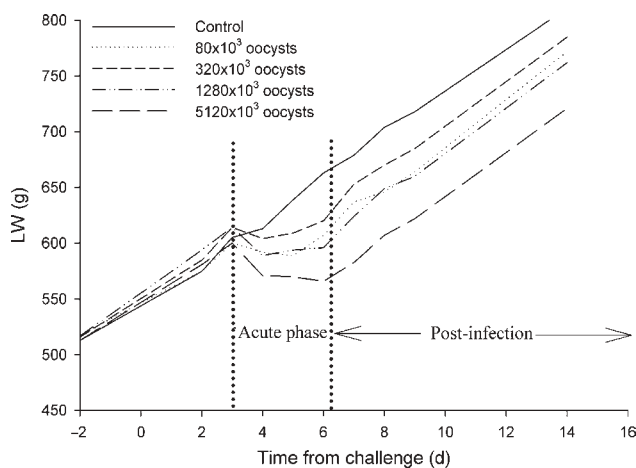

Figure 1 The effect of different single challenge doses of the protozoan *Eimeria acervulina* on the live weight, *LW* g, of chicks over the time course of an experiment, which included the acute- and post-infection phase (Hein, 1968).

response experiments where challenged animals and healthy (pair-fed) controls are given foods with different protein contents. On the other hand, it could be possible to use an infusion approach with different levels of nutrient infusion and where nitrogen balance is measured over the entire period of an infection. Future modelling attempts, using either mechanism, may contribute towards our understanding of how growth is reduced during pathogen challenges.

Effects of pathogen challenges on maintenance. Marginal response experiments were identified for five different kinds of 'pathogen challenges': a 'high immune system activation' treatment of pigs (Williams *et al.*, 1997a, b and c), a bacterial antigen challenge of chicks (*LPS*, Webel *et al.*, 1998a and b), challenges with gastro-intestinal parasites of chicks (*Eimeria acervulina*, Willis and Baker 1981a and b) and sheep (*Trichostrongylus colubriformis*, Poppi *et al.*, 1986, Datta *et al.*, 1998) and a challenge of pigs with blood borne parasites (*Trypanosoma vivax*, Fagbemi *et al.*, 1990; Otesile *et al.*, 1991). Data were analysed using either linear or continuous linear plateau regressions.

The experiments of Williams *et al.* (1997a, b and c) and Webel *et al.* (1998a and b) were the only experiments found in the literature, which measured *PR* in relation to different levels of protein or amino acid intake during a 'challenge'. In the experiments of Williams *et al.* (1997a, b and c), foods with different crude protein contents were used (designed with lysine as the first limiting amino acid). Webel *et al.* (1998a and b) formulated diets to provide different levels of lysine, threonine or arginine. The response in *PR* to lysine intake is shown in Figure 2, for chicks challenged with *LPS*, with a continuous linear plateau model (described in Sandberg *et al.* (2005b)) fitted to the data.

The marginal response in Figure 2 was not different, while maintenance increased slightly, and the challenged
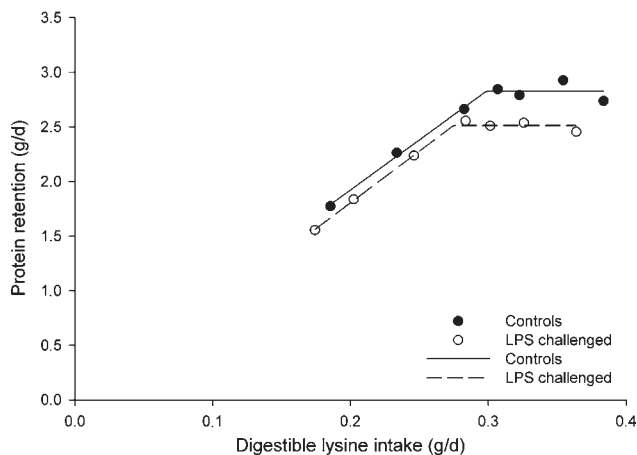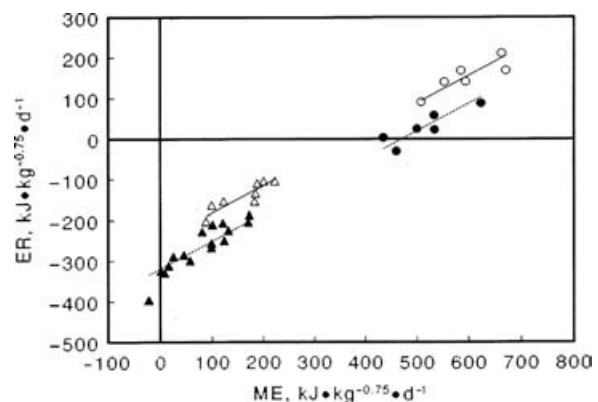
animals did not achieve the same maximum rate of *PR*, as discussed earlier. The response varied with different amino acids. Maintenance in the challenged animals varied from 1 to 1.3 times that in the healthy. Webel *et al.* (1998a and b) found that chicks supplemented with lysine had an increased maintenance, while those with threonine tended towards an increase, with no effect for arginine. Different effects on maintenance may suggest that amino acid requirements were affected to different extents. LPS stimulates an acute phase response, including the production of acute phase proteins, which have different amino acid contents from body protein retained in the healthy animal (see Table 1). The experiments of Webel *et al.* (1998a and b) also suggest that it may be necessary to consider the requirements of individual amino acids to fully account for reductions in growth during pathogen challenges.

The experiments of Williams *et al.* (1997a, b and c) agree with those of Webel *et al.* (1998a and b), but with greater maintenance levels in two cases (2.9 and 1.6 times their controls). In both sets of experiments, however, the challenges used would be expected to have a small to moderate effect on the host, compared with actual bacterial, viral or parasitic challenges. To describe the effects of a particular pathogen on requirements for maintenance it is necessary to perform experiments that account for the entire time course of an infection. Marginal responses that are measured over the entire time course of defined pathogen challenges, and in particular during the acute phase (as shown in Figure 1) of an infection, at different doses would allow this.

Marginal response experiments with limiting energy have shown increased maintenance requirements as determined by regressions of live weight gain against energy intake, Figure 3. West African Dwarf goats challenged by *Trypanosoma vivax* (Van Dam, 1996) had approximately

Figure 2 The response in protein retention (g/day) to digestible lysine intake (g/day) of chicks challenged with LPS on every 2nd day over an 11-day period, in relation to their unchallenged controls (data from Webel *et al.* (1998a)). A continuous-linear-plateau model (described in Sandberg *et al.* (2005b)) was fitted to the data.

Figure 3 The marginal response in energy retention (ER) to metabolisable energy intake (ME) for West African Dwarf goats challenged with *Trypanosoma vivax*; figure reproduced from Van Dam (1996) with permission. Regressions fitted with a common slope; open symbols are the non-infected, and closed symbols are the infected goats: the circles and triangles make the distinction between two different quality (low and high energy content) foods.

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maintenance requirements 1.2 times as great as their healthy counterparts.

Experiments with pigs are in further support of an increase in maintenance requirements for energy (Fagbemi *et al.*, 1990; Otesile *et al.*, 1991). In these experiments the pigs were challenged with the same dose of *Trypanosoma brucei* at two different live weights. The 10 kg pigs (Fagbemi *et al.*, 1990) had a greater increase in energy maintenance requirements (2.2 times healthy controls) than 100 kg pigs (Otesile *et al.*, 1991), which had maintenance requirements 1.7 times of healthy controls. This suggests that animals of different sizes may have different changes in requirements at a challenge dose, but the issue that remains is how to relate pathogen challenges to size.

The effects of different challenge doses on hosts of different sizes need to be accounted for in both predictive frameworks and in the design of experiments. The issue is in two parts: (1) what is the relevant measure of animal size when quantifying the effects of pathogen challenges; (2) is it necessary to take into account, not only animal size, but also an animal's degree of maturity (or age) through some scaling rule? No clear data exist which allows for a choice to be made between body weight, protein weight and protein weight combined with a measure of fatness, as a measure of size. The second issue is even more difficult, and experiments that provide sufficient data to test it are needed. Satisfactory quantification of a pathogen challenge becomes crucial when considering interactions between dose, genotype and nutrition on the outcome of pathogen challenges. This has not been considered in a large number of experiments that address these interactions.

Effects of pathogen challenges on the marginal response. Pathogen challenges have been found to have different effects on the marginal response in growth for different pathogens. Williams *et al.* (1997a, b and c) and Webel *et al.* (1998a and b) found no effect of high immune system activation and *LPS*, respectively, on the marginal response in *PR* to either protein or amino acid intake. Willis and Baker (1981a and b), using a specific pathogen challenge (*Eimeria acervulina*) at different doses on chicks found a slight effect on the marginal responses in live-weight gain to amino acid intake at a larger dose. The slope for chicks challenged with 2×10^5 oocytes was reduced numerically by 10%, while with a challenge of 1×10^6 oocytes the slope was reduced significantly by 22%. Taken together, however, marginal response experiments in monogastrics may suggest little effect on the marginal material efficiency of growth.

Experiments with sheep where either live weight gain or nitrogen retention were regressed on *FI* suggested an effect on the marginal response (Poppi *et al.*, 1986; Datta *et al.*, 1998). The marginal response of the sheep was not affected during the initial weeks of a continuous challenge. Around the time when the sheep were likely to have had the greatest worm burdens and just prior to starting to express immunity, the slope was reduced and the intercept displaced, indicating a reduced marginal response and an increase in maintenance. The marginal response was no different from that of their controls once the animals had acquired immunity, and, overcame the challenge (Poppi *et al.*, 1986; Datta *et al.*, 1998).

The time dependent effect on the slope as suggested by Poppi *et al.* (1986) appears to coincide with the accumulation of the worm burden for that pathogen e.g. Van Houtert *et al.* (1995). These time (pathogen load) dependent effects on the marginal response may explain why Webel *et al.* (1998a and b) and Williams *et al.* (1997a, b and c) did not find any effects on the slope. *LPS* challenges have short-term effects, and Williams *et al.* (1997a, b and c) used an undefined 'high immune system activation'. To predict the effects of pathogen challenges on marginal responses in growth to resource intake it may be necessary to account for the kind and dose of pathogen together with the development of a challenge over time. The effect of pathogen challenges on the marginal response in growth, however, would appear to be small in magnitude.

The causes of increased requirements during pathogen challenges

Pathogen challenges cause increased requirements for both protein and energy, and these appear to be variable for different kinds and doses of pathogens. In addition to marginal response studies, it may be possible to quantify the increased requirements by considering other kinds of evidence. Such evidence provides estimates of the protein and energy costs of specific consequences of pathogen challenges. The quantitatively more important consequences of pathogen challenges include mounting an immune response, repairing damaged tissues and replacing lost fluids, and mounting a fever. In principle, such evidence may be combined with that considered in earlier sections to provide a clearer description of the nutritional costs that are associated with pathogen challenges.

The protein cost of an immune response
Attempts have been made to quantify the specific protein cost of mounting an immune response either by trying to sum up the masses of immune proteins (Klasing and Calvert, 1999; Houdijk *et al.*, 2001), or through direct experimentation (Pilorz *et al.*, 2005). It is difficult to derive such a cost, as the immune response consists of several different components, many of which are expressed differently over the time course of a pathogen challenge. Hosts may respond with innate or acquired immunity, or a combination of these, depending on the level of challenge and the stage of a particular infection (Heegard *et al.*, 1998; Klasing, 1998; Taylor-Robinson, 2000). An approximation of the protein cost of mounting an immune response, however, could provide an initial estimate that could be

included in a predictive framework of resource requirements and partitioning during pathogen challenges.

Klasing and Calvert (1999) attempted to sum up the amounts of different immune proteins of innate and acquired immune functions to estimate the cost (in terms of lysine) of mounting an immune response. The lysine cost for the immune response was equivalent to a reduction in growth of 0.031 g ADG per kg BW per day; this would approximate to 0.0015 g of protein ($0.031 \times 0.3 \times 0.16$) for a 0.3 kg chick. Klasing et al. (1987) challenged chicks with LPS and found a reduction in body growth rate of 5.7 g/day compared with pair-fed controls. In part, some of this reduction in growth rate would have been associated with the cost of mounting a fever as reported by the authors. Their finding does suggest a larger cost for producing immune proteins ($0.16 \times 5.7 = 0.9$ g/day) than that predicted by Klasing and Calvert (1999).

The marginal response experiment of Webel et al. (1998a and b) suggested that chicks challenged by LPS had an increase in maintenance requirements for certain amino acids. Their challenge would have mainly stimulated an innate immune response, e.g. the production of acute phase proteins. LPS challenges would not result in a cost of damage to the host, as may be the case for 'real' bacterial challenges (Wannemacher et al., 1971; Powanda et al., 1972; Wannemacher et al., 1974). Thus, the increase of 1.3 times the maintenance requirement of the healthy animal (Webel et al., 1998b) is indicative of the cost of an innate immune response. The ideal protein requirement for maintenance, MP g/day, for a healthy 0.3 kg chick could be estimated as 0.26 g/day from the equations of Emmans and Fisher (1986). A 1.3 times increase in maintenance would then lead to the average cost for mounting an innate immune response to be estimated as 0.08 g/day of ideal protein (or 0.27 g ideal protein per kg BW), assuming an efficiency of 1. This cost is sufficiently large to warrant inclusion in a framework of protein (amino acid) requirements and partitioning.

Houdijk et al. (2001) attempted to estimate the metabolisable protein cost for sheep expressing immunity to a gastro-intestinal parasite. The sum of increased cell flow in lymph, flow of IgA, production of mucosal mast cells and sheep mast cell proteases was 52.8 mg/kg $BW^{0.75}$ per day: this would be 0.02 g of metabolisable protein for a 0.3 kg animal, or 0.07 g MP per kg. Scrimshaw (1991) based on nitrogen balance experiments of humans proposed much greater requirements for protein which included all consequences of a pathogen challenge. An average estimate for infections ranging in severity was proposed as 0.57 CP per kg per day, which could rise to 1.2 g CP per kg per day for more severe infections. The approach taken by Houdijk et al. (2001) and Klasing and Calvert (1999) were restricted by it effectively being impossible to sum up the production of all immune proteins during a pathogen challenge. An underestimation may thus occur by not accounting for all immune proteins, not accounting for greater requirements for certain amino acids than others, through

an effect on protein (amino acid) partitioning, or a combination of these.

There is other evidence in favour of significant requirements for mounting an immune response. A series of experiments were performed at the United States Army Medical Research Institute of Infectious diseases e.g. Powanda et al. (1972), Thompson and Wannemacher (1973), Wannemacher et al. (1971 and 1974) and Berendt et al. (1977) using a rodent model. In all of their experiments, which mainly included bacterial challenges, large increases occurred in the uptake of certain radioactively labelled amino acids by the liver; this lead to a reduced uptake of amino acids by muscle. Assuming that the increased uptake is due to the production of immune proteins, Thompson and Wannemacher (1973) estimated that the amino acid uptake of the liver had increased by 1.38 times that of pair-fed controls. The increases coincided with drops of certain free amino acids in blood, which also appeared to coincide with the growth and decay of the pathogen load in the host (Klebsiella pneumoniae, Berendt et al., 1977). Further support for a host mounting an immune response that is proportional to its pathogen load has also been shown for Plasmodium vivax (Taylor-Robinson, 2000).

Experiments are warranted that measure the marginal response using a suitable methodology (Batterham et al., 1990; Chung and Baker, 1992) at different time points of a pathogen challenge for different amino acids. Such experiments may enhance our current estimates of the amino acid requirements during expression of both innate and acquired immune responses. It would also be necessary to measure the development of the pathogen challenge in the host, together with indicators of immune responses. While such experiments are complex and expensive, they may significantly contribute to the lacking evidence base for amino acid requirements during pathogen challenges.

Repair and replacement of damaged tissues and body fluids

Pathogens may cause damage to a host's tissues e.g. gut wall or specific cells e.g. red blood cells and cause body fluids to leave their natural compartments such as plasma leaking into the GIT (Abbott et al., 1985; Yu et al., 2000). The animal would need to repair such damage or replace lost fluids to maintain normal function, which is thus a direct cost to the animal (Berendt et al., 1977). It would be expected that such costs are larger than those associated with expression of immunity. The factors that may need describing fully to account for this cost include the type of pathogen, level of challenge and ability of the host to withstand a particular challenge.

Parasitic worms used in the experiments summarised by Hale (1985) affected the stomach (Hyostrongylus rubidus), small intestine (Strongyloides ransomi), large intestine (Oesophagostomum. spp and Trichuris suis) and the kidneys (Stephanus dentatus). In a number of cases, the reduction in nitrogen retention was explained by reduced

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nitrogen intake (due to anorexia) combined with increased nitrogen in the faeces. The additional nitrogen in the faeces would be likely to have been associated with damaged tissues and endogenous secretions. These effects were noted to the greatest extent for challenges of the small and large intestine. The kidney parasite, at the dose used, caused a reduction in nitrogen retention that was only associated with the reduction in *VFI*. Findings for gastro-intestinal parasites in sheep (Sykes and Coop, 1977; Kimambo *et al.*, 1988) agree that level of cost associated with damage depends on the location of the gut that is affected.

Literature data shows that the extent of the costs associated with damage is not only dependent on the pathogen species but also on level of challenge. Le Jambre (1995) considered the relationship between blood loss and estimated worm numbers (pathogen load) of *Haemonchus contortus* in the abomasum of sheep. The pathogen causes blood loss by direct sucking of blood and from a short period of leaking once the parasite has detached. Le Jambre (1995) found that the relationship between blood loss and worm numbers was of an exponential form. This may indicate that a host that has already suffered a certain amount of damage may be less able to deal with the pathogen challenge. Beer *et al.* (1974) also found that erythrocyte losses into the GIT of pigs challenged by three different levels of *Trichuris suis* were directly related to the level of challenge.

Powanda *et al.* (1975) measured the pathogen load of *Francisella tularensis* and the extent of liver damage in rats: the form of the relationship was the same as that found for *Haemonchus contortus* by Le Jambre (1995). Thus, the relationship between damage and pathogen load may be general for different pathogens, but quantitatively different. A challenge with *Actinobacillus pleuropneumoniae,* which causes lesions of the lungs, have also shown that during the early stages of infections lesions develop and once the animals reach slaughter these are much less abundant or absent (Magnusson *et al.*, 1997; Black *et al.*, 1999).

The extent of plasma loss has been estimated in a number of experiments over the time course of challenges of sheep with different doses of gastro-intestinal parasites. Data of plasma loss from Steel *et al.* (1980) were used to estimate protein requirements associated with plasma loss for different doses of *Trichostrongylus colubriformis*. It was assumed that plasma contains 75 g protein per l (Houdijk *et al.*, 2001). The protein requirement due to plasma loss was also directly related to the level of challenge, and the form of the response appears to be similar to how the pathogen load (or worm burden) develops in the host. Symons *et al.* (1981) estimated the plasma loss due to a challenge of sheep with *Teladorsagia circumcincta* was an approximation, as it is difficult to the estimate the extent to which plasma is reabsorbed. While the relationship between the different doses were not as clear as those observed for *Trichostrongylus colubriformis* by Steel *et al.*

(1980), the total quantity of protein lost was quite similar. The average protein requirement due to plasma loss was calculated as 9.6, 12.4 and 10.4 g protein per day for challenge doses of 12 000, 37 500 and 120000 of *Teladorsagia circumcincta* larvae, respectively. These are very significant costs to the animal.

Data from challenges with gastro-intestinal parasites and bacteria show that the repair and replacement of damaged tissues and lost fluids may be a significant cost to the animal. Pathogen induced damage appears to be of a general kind for different kinds of pathogens; the extent of the damage, at a pathogen load, will depend on pathogen species and virulence (Willis and Baker, 1981a). Therefore, to predict the extent of damage it is necessary to account for pathogen kind, dose and virulence. The ability of the host to overcome the infection and prevent damage to be caused would also need to be considered. The interactions between pathogen kind, host genetic resistance and nutrition (see below) on the extent of damage could then be explored.

Requirements for energy during pathogen challenges
It has been proposed that a pathogen challenge may cause increased requirements for energy due to the production of immune proteins (Demas *et al.*, 1997), additional nitrogen processing (Sykes and Coop, 1977) and especially expression of fever (Baracos *et al.*, 1987). In the framework considered here any energy costs associated with the production of immune proteins could be incorporated within the overall cost of protein production, while other costs would need accounting separately. The current quantitative evidence of these costs is now considered.

Non-pathogenic antigen challenges. It is difficult to estimate the energy cost that is associated with the production of only immune proteins. The model used by several authors e.g. Demas *et al.* (1997), Mashaly *et al.* (2000), Pilorz *et al.* (2005) is challenges with non-pathogenic antigens such as sheep red blood cells or keyhole limpet hemocyanin (an immune stimulant glucoprotein). In principle, any effects on energy metabolism would be the consequence of the expression of immune proteins and not due to repair of damage or fever.

Several experiments were identified in which total energy expenditure was measured in-directly through measurements of oxygen consumption during challenges with non-pathogenic antigens. Any difference in energy expenditure is then estimated roughly from the rate of oxygen consumption (20.1 kJ/l O_2; Eraud *et al.* (2005)) between challenged and non-challenged animals, which are shown in Table 2.

The data in Table 2 show that a significant energy requirement is associated with the production of immune proteins, given that any changes in energy expenditure are due to antibody production. The relative changes in energy expenditure appear to be within a similar range, except of

Table 2 *The relative change in energy expenditure (RCE, challenged/controls) and energetic cost (E$_{antibody}$, challenged energy expenditure − control energy expenditure) due to antibody production, as measured by changes in oxygen consumption of animals and birds challenged with non-pathogenic antigens*

Host	Body weight (g)	RCE	E$_{antibody}$ (kJ/kg BW per day)	RCE proportional to antibody production	Source
Mice[†]	36.0	1.55	230	Yes	Demas *et al.* (1997)
Blue tits	10.0[‡]	1.10	275	Yes	Svensson *et al.* (1998)
Great tits	18.6	1.09	148	−	Ots *et al.* (2001)
House sparrows	28.4	1.29	148	−	Martin *et al.* (2002)
Collared doves	130	1.09	36	Yes	Eraud *et al.* (2005)

[†] The antigen used was keyhole limpet hemocyanin (an immune stimulant glucoprotein) in all other cases it was sheep red blood cells.
[‡] Estimated from age.

that of Demas *et al.* (1997): this may be explained by the use of a different antigen, which induced an increase (>1°C) in colonic temperature. Demas *et al.* (1997), Svensson *et al.* (1998) and Eraud *et al.* (2005) found that the largest increase in energy expenditure coincided with peak antibody production. It would appear, therefore, that the energy cost of mounting an immune response is strongly related to the level of the immune response. This in turn may explain why some authors have not found any effects on overall energy expenditure or growth (Henken and Brandsma, 1982; Pilorz *et al.*, 2005).

Difficulties may also arise from other components of an animal's energy budget being affected. Even a non-pathogenic antigen challenge (such as sheep red blood cells) may reduce the energy expenditure associated with activity (Henken and Brandsma, 1982) as has been observed for pathogenic challenges (Van Diemen *et al.*, 1995; Van Dam, 1996). Energy requirements due to production of immune proteins may therefore be partially 'hidden' within the overall energy budget of the animal. Experiments that have tried to consider the individual components of an animal's energy budget are now considered to further characterise the effects of pathogen challenges on energy requirements.

Energy balances, marginal responses and fever. Experiments with both pathogenic antigen challenges and challenges with live pathogens have shown maintenance requirements to be 1.05 to 1.35 times as great. There appears to be highly specific pathogen differences, with the greatest energetic cost occurring for pathogen or antigen challenges that lead to a fever e.g. Verstegen *et al.* (1991), Zwart *et al.* (1991) and Demas *et al.* (1997). Local pathogen challenges may cause less of an effect as was demonstrated by Van Diemen *et al.* (1995) who considered the effect of atrophic rhinitis on heat production in pigs.

Gastro-intestinal parasites may cause an increase in energy requirements through the repair of damaged tissues in the gut, or through the reprocessing of nitrogen leaked into the GIT that appears to later be partly reabsorbed (Poppi *et al.*, 1986). MacRae *et al.* (1979) performed both energy and nitrogen balances of sheep, at different time points of a challenge with *Trichostrongylus colubriformis*. No differences were observed prior to week 4 between challenged sheep and their pair-fed controls, after which the metabolisability (ME/GE) was reduced compared with pair-fed controls, as is shown in Figure 4 together with their faecal egg counts.

This suggests a significant increase in excretion of energy either in the urine or faeces, which is associated with the peak of the pathogen challenge; as in later balance periods there was no difference in metabolisability. As was the case for the nitrogen cost of damage, it appears necessary to predict the energetic cost of repairing damaged tissues, which appears to be related to the level of challenge of the host.

A significant body of evidence exists from experiments with *Trypanosoma vivax* infection in West African goats (Verstegen *et al.*, 1991; Zwart *et al.*, 1991; Van Dam, 1996; Van Dam *et al.*, 1997 and 1998) on both energy and nitrogen requirements. *Trypanosoma vivax* in these experiments caused an increase in body temperature (consistently 0.8 to 1°C) and damage to red blood cells (Van Dam, 1996). Energy requirement for maintenance was between 1.15-1.27 times that of healthy control animals. The findings of Van Dam (1996) and Van Dam *et al.* (1997) are also shown in Figure 3.

The above changes in energy expenditure are likely to be underestimates as *FI* was reduced in all cases and the goats showed behavioural coping responses (Van Dam, 1996), which may have further masked the energetic cost. The cost due to fever has been shown to differ for goats that were either standing or lying (Van Dam, 1996). The most crucial finding, however, is that of Zwart *et al.* (1991) who found that an increase in heat production due to fever is different, when measured during either day or night. Van Diemen *et al.* (1995) also found time dependent effects on heat production in pigs challenged by *Pasteurella multocida*. This is important for future experiments that attempt to quantify the energetic cost of pathogen challenges. If heat production is measured over a sample period during the day (a common practice) then the cost due to fever will be underestimated. Marginal response experiments with pigs are in support of increases in maintenance requirement by 1.7 and 2.2 times in pigs challenged with a *Trypanosome* (Fagbemi *et al.*, 1991; Otesile *et al.*, 1991).
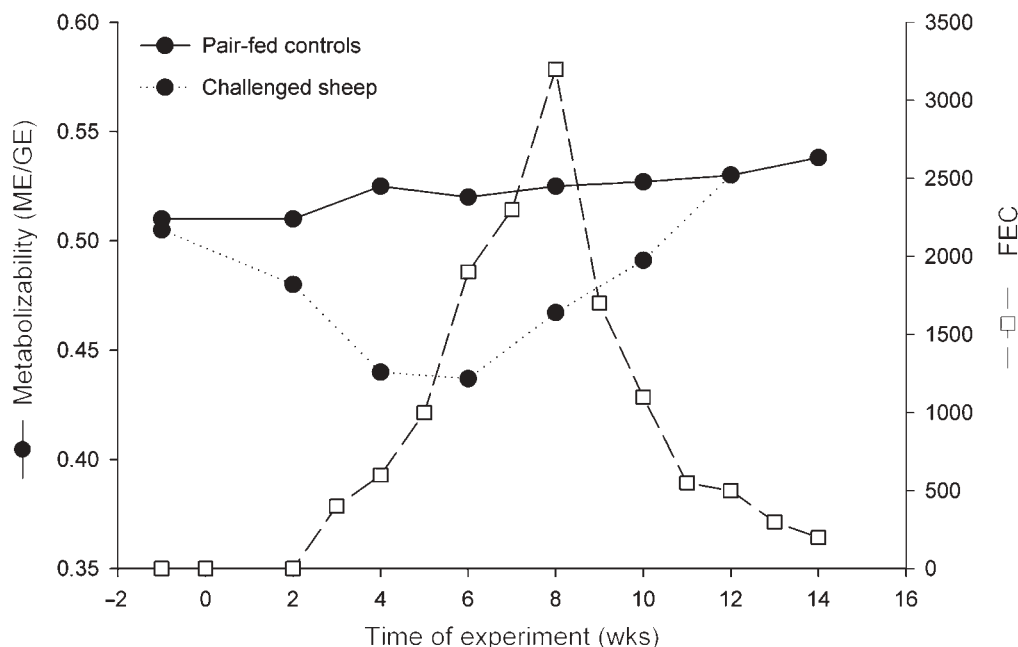
Figure 4 The relationship between food metabolisability (ME/GE), faecal egg counts (FEC) and time of a sub-clinical trickle infection of sheep with the intestinal worm *Trichostrongylus colubriformis* (MacRae *et al.*, 1982).

Experiments with other pathogens appear consistent with those using the Dwarf goat-*Trypanosoma vivax* model in terms of the energetic cost of fever. Baracos *et al.* (1987) reviewed the energy requirements caused by fever and concluded that a 1°C increase in body temperature due to fever, gave an energy requirement of 1.1 times as much. It was discussed, however, that the effect of fever on metabolic rate was time dependent, which is likely explained by its close relationship to the pathogen load in a host. Different types of antigens (either *LPS* or heat killed *Staphylococcus areus*), however, may cause different energy requirements (Benson *et al.*, 1993), as chicks challenged with *LPS* grew much less lipid than controls or chickens challenged with heat killed *S. aureus*. A predictive framework would need to consider the relationship between fever and pathogen challenge (kind, dose and virulence) and have clear definitions of how to include other energetic costs such as production of immune proteins or repair of damage within the overall energy budget of a host.

Partitioning of scarce protein and energy resources during pathogen challenges

The food intake of an animal may be less than its desired intake i.e. that which permits maximal growth, due to the nature of the food, the environment (the animal is hot) or pathogen induced anorexia. The key to predicting growth in such circumstances is to have a suitable partitioning rule. Sandberg *et al.* (2005a and b) argued that e_p (the marginal material efficiency of protein retention) is central to the overall system of (protein and energy) partitioning as once protein retention has been predicted, lipid

retention can be accounted for on energy grounds e.g. Emmans (1994). The problem is whether pathogen challenges lead to a different overall system of partitioning. This has been considered qualitatively by Beisel (1977), Van Houtert and Sykes (1996), Coop and Kyriazakis (1999), Klasing and Calvert (1999) and Lochmiller and Deerenberg (2000) amongst others. The focus here is to bring together suitable quantitative evidence of both protein (amino acid) and energy partitioning to assess the largely qualitative partitioning rules that have been proposed during disease.

Rules of partitioning during pathogen challenges
Partitioning rules that have been proposed during pathogen challenges have either considered resource supply in terms of protein (Coop and Kyriazakis, 1999), amino acids (Koutsos and Klasing, 2001; Le Floc'h, 2004) or energy (Schrama *et al.*, 1997). With the exception of Coop and Kyriazakis (1999) partitioning of resources between different arms (innate or acquired) of immune responses has not been considered. It may be necessary to distinguish between different immune responses: the most attractive solution would be where all consequences of a pathogen challenge could be considered as one source for allocation. Two types of partitioning rules are considered here. The classical view is where requirements for maintenance are met first (including costs associated with pathogen challenges), after which resources are partitioned to growth (Klasing *et al.*, 1987; Schrama *et al.*, 1997; Verhulst *et al.*, 1999; Elsasser *et al.*, 2000; Lochmiller and Deerenberg, 2001; Colditz, 2002). Alternatively it has been suggested that there is a degree of competition between growth and immune functions once the requirements for maintenance (which may still include some consequences of disease)

have been met (Coop and Kyriazakis, 1999), when nutrient and energy resources are scarce.

The consequence of classical partitioning is that for intakes above maintenance no further improvements in immune responses would be observed. There are several experiments to contradict this, including those of Bhargava *et al.* (1970a and b), Datta *et al.* (1998) and Lee *et al.* (2002). Some authors propose that pathogen challenges always lead to a release of amino acids from body protein stores such as muscle e.g. Klasing *et al.* (1987) and Elsasser *et al.* (2000). This would be likely to be the case for sub-maintenance intakes, (i.e. the animal could still maintain some small degree of immunity). It is unlikely, however, that an animal would preferentially utilise body protein when dietary resources are available. This would lead to a fitness penalty in the long term (Coop and Kyriazakis, 1999). Thus, it is necessary to consider the quantitative evidence of partial protein (amino acid) partitioning between growth, consequences of pathogen challenges and immune functions.

During pathogen challenges, energy requirements for maintenance are increased, with no further effect on energy partitioning e.g. Benson *et al.* (1993) and Van Dam (1996). It has been observed that during pathogen challenges heat production was increased, while food intake and energy retention were reduced e.g. Verstegen *et al.* (1991) and Zwart *et al.* (1991). This effect, however, appears to be both pathogen and dose specific. Takhar and Farrell (1979a) found that a challenge with *Eimeria acervulina* did not affect the heat production of chicks, compared with their pair-fed controls. In a predictive framework energy requirements during pathogen challenges could therefore be accounted for as part of maintenance and the cost of protein retention, and any shortfalls would be met by using energy stores in the form of lipid. This would have the consequence that only protein partitioning during health would need to be revised in a predictive framework of growth during disease. In support are the several experiments that show no improvement in immune responses when additional levels of energy are supplied e.g. Van Heugten *et al.* (1996) and Spurlock *et al.* (1997). Therefore, only protein (amino acid) partitioning during pathogen challenges are now considered.

Quantitative assessment of protein partitioning during pathogen challenges
The experiments of Williams *et al.* (1997a, b and c) and Webel *et al.* (1998a and b), described earlier, found no effect on the marginal responses in protein retention of growing pigs and chicks, respectively, to protein and amino acid supplies. A possible interpretation is that as marginal response in protein (amino acid) retention to protein (amino acid supplies) was not different, this leads to the rejection of partial prioritisation of protein between growth and immune responses. Another interpretation is that the partitioning of protein between growth and immune responses could not be detected in these experiments when measuring overall protein retention. Protein that is partitioned towards growth and immune responses are both retained in the body. Any net benefit that is observed in terms of overall growth rate, may only be due to an alleviation of damage caused by the pathogen e.g. Abbott *et al.* (1985) and in particular Datta *et al.* (1998). The relationships between level of nutrition, innate or acquired immune responses and damage are now considered.

Innate immune responses. The immune proteins that are produced during the acute phase of an infection, such as the acute phase proteins and complement are here defined as innate immune proteins. It is recognised that the acute phase proteins have multiple functions, which include supporting the acquired arm of the immune response and assisting in the repair of damaged tissue (Murata *et al.*, 2004). The question is whether these immune functions are affected by amino acid availability, in animals that are growing.

Dritz *et al.* (1996) found no significant effect of three different foods on the expression of the acute phase protein haptoglobin in pigs that were gaining weight. Sakamoto *et al.* (1998) reviewed evidence of how nutrition may affect another set of innate immune proteins (complement) and did not find any effects of nutrition in 'normal' animals. In malnourished animals, however, it would appear that an additional supply of resources may affect the expression of the complement system, but this was only the case for severely malnourished humans. Fleck (1989) stated that "Prolonged protein-energy depletion in man sufficient to yield a decrease in body weight of 25% led to only a 7% decrease in concentration of albumin...When the increase in plasma volume is taken into account there was no change in the amount of albumin in the circulation". It would appear, therefore, that costs relating to innate immune responses may need including as part of the requirements for maintenance. The marginal response studies of Webel *et al.* (1998a and b) are in support as they used *LPS* as an antigen, which largely stimulates an acute phase response.

Acquired immunity. Coop and Kyriazakis (1999 and 2004) reviewed information in the literature on the effects of protein supplementation on immune responses and growth in sheep challenged with gastro-intestinal parasites. They concluded that the expression of immunity was affected by nutrition from experiments with sheep (Bown *et al.*, 1991; Kambara *et al.*, 1993), cattle (Mansour *et al.*, 1991 and 1992) and goats (Singh *et al.*, 1995). There is also similar evidence in pigs (Van Heugten *et al.*, 1996) and mice e.g. Ing *et al.* (2000). Other kinds of pathogen challenges were considered here to determine whether the improvement of the expression of acquired immunity is a general response across different types of pathogens.
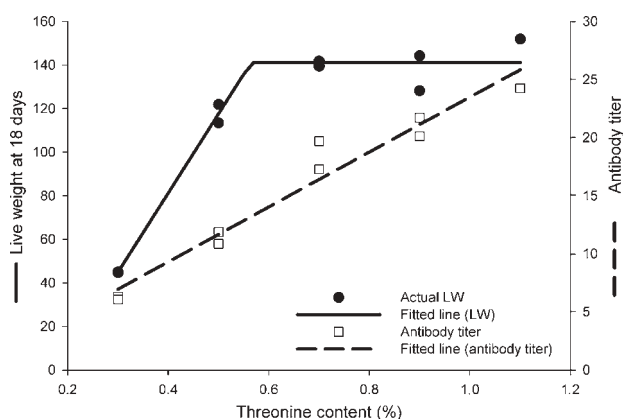
Tsiagbe *et al.* (1987) found that growth and immune responses (antibody expression) of chicks were increased with increasing levels of methionine supplementation.

Antibody expression was only improved once a plateau had been achieved in growth rate, thus their data suggest that growth was prioritised over immune responses. Bhargava *et al.* (1970b), on the other hand, found that chicks challenged with the Newcastle virus had improved growth rates and geometric mean antibody production when given foods that had increasing proportions of L-threonine. This suggests a partial prioritisation of amino acid between growth and immune responses, shown in Figure 5.
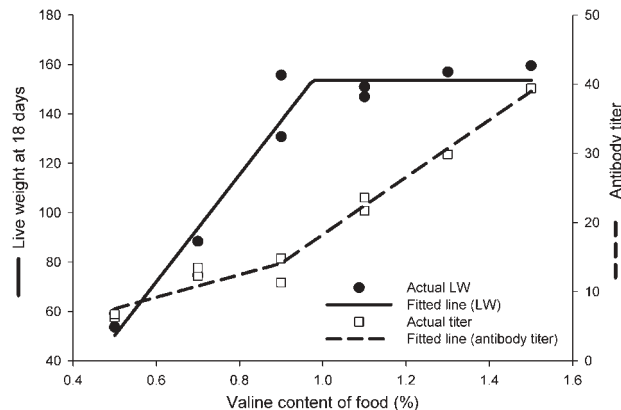
Figure 5 does suggest that as the animals reached a plateau in growth there was no distinct improvement in antibody titer, which would not be expected if protein was being partitioned between these two functions. Bhargava *et al.* (1970a), however, found a different response for chicks challenged with Newcastle virus and given food of different amino acid (valine) contents, shown in Figure 6. In this case, the response in antibody titer did further increase when the animal reached a plateau in terms of growth, and thus providing strong evidence that valine was being partitioned between growth and immune functions.

The data in Figure 6 are in support of a partial prioritisation of additional levels of amino acid supply between growth and immune responses. Furthermore, these two experiments indicate that the requirements of specific amino acids and their partitioning need accounting for in predicting growth and immune responses. The level of challenge may affect the extent to which protein (amino acid) is partitioned between growing 'body' protein and immune proteins (Wannemacher *et al.*, 1974).

There are several publications in favour of additional levels of protein of amino acid supplementation having a beneficial effect on the host coping with several different kinds of pathogen challenges. Lee *et al.* (2002), for example, found that arginine supplementation (a limiting amino acid in birds) improved immune responses in chicks

Figure 6 The responses in live weight over 18 days (LW) and antibody titres (AT) to increasing valine contents (V) of a food for chicks challenged with a Newcastle virus from Bhargava *et al.* (1970a). Linear plateau response was approximated as a linear function of valine content (LW $= 217.V - 58.2$) until the plateau of 153.6 was reached. The linear regressions of antibody titre against valine content until maximum LW was reached was AT $= 16.37.V - 0.6325$ whereas for the subsequent phase was AT $= 41.35.V - 23.04$.

challenged with the infectious bronchitis virus. Low *et al.* (2003) found that mice given a whey protein based diet, which is rich in certain amino acids found in large amounts in immune proteins, had increased antibody responses to several bacterial and viral antigens.

The likely explanation for hosts coping better with pathogen challenges when provided with sufficient amounts of protein (amino acid) is the control and elimination of the pathogen from the host (Van Houtert *et al.*, 1995). The level of pathogen load (in particular the maximum pathogen load) is directly related with survival e.g. Powanda *et al.* (1975) and Berendt *et al.* (1977). Suzuki *et al.* (1993) found increased survival in mice challenged with *Staphylococcus aureus* when given glutamine supplementation. Unfortunately, the change in pathogen load and performance over time for different bacterial and viral pathogens has not been measured extensively, whilst nutrition has been manipulated. Sheep challenged with gastro-intestinal parasites show reductions in pathogen load (worm burden) when given additional levels of protein (Van Houtert *et al.*, 1995; Donaldson *et al.*, 2001) or amino acid (methionine: Miller *et al.* (2000)) supply. In most of these cases relatively low levels of protein supply (compared with those required for maximal growth) were required to achieve such responses. The quantitative relationship between degree of resource scarcity and rate of pathogen removal is thus central to predicting performance during pathogen challenges.

The problem of predicting resource partitioning during pathogen challenges appears to consist of three sub-problems. Firstly, it is necessary to predict the partitioning of amino acids between growing 'body' and immune proteins during exposure to different levels and kinds of pathogens. Secondly, it would be necessary to be able to account for the net benefit an animal would gain from investing in an

Figure 5 The live weight at 18 days (LW) and geometric mean antibody titres (AT) for chicks challenged with a Newcastle virus and given foods that had different contents of L-threonine from Bhargava *et al.* (1970b). Linear plateau response was approximated as a linear function of threonine content for live-weight gain (LWG $= 364.T - 64.4$) until the plateau of 141.1 was reached. The linear regression of antibody titer against valine content was equal to AT $= 23.63T - 0.143$.

immune response. Finally, a predictive framework would need to adequately describe how different genotypes partition their resources between growth and immune functions. This would allow consequences of genetic selection strategies (i.e. increased potential for growth or resistance to pathogens) to be explored, and for management (nutrition) strategies to be better informed.

Discussion

Improving current predictive growth models will help to inform genetic selection and management strategies intended to combat the effects of disease on animal performance and improve their health and welfare. This has become increasingly important, as pathogens have evolved resistance to pharmaceutical interventions (Waller, 1997), antimicrobial growth promoters have been banned (EC Regulations 1831/2003, 1834/2003) and animal welfare (including health) has become more central to animal production (Kanis et al., 2005). No comprehensive attempt has been made to model the effects of disease on growth and performance. Black et al. (1999) when discussing the inclusion of disease in the AUSPIG model (Black et al., 1986) suggested that "…increasing maintenance energy requirements by up to 1.3 times the normal predictive value, decreasing the rate of protein deposition by 0.9 times normal and decreasing feed intake down to zero depending on the severity and duration of the disease" would achieve this. This quasi-quantitative suggestion might be a good first step. The aim here was to extend the description of the problem of predicting growth during pathogen challenges; issues that have not yet been considered are now discussed.

It appears from literature evidence that energy partitioning during pathogen challenges might remain the same as that during health, with any effects of pathogen challenges on energy requirements being included as part of maintenance. During bacterial, viral and some parasitic pathogen challenges it is common for an animal to mount a fever (Hart, 1988; Blatteis, 2003). A febrile response (within a certain range) may be seen as a beneficial host reaction, rather than being a detrimental and unavoidable consequence of pathogen challenges e.g. Hart, 1988, Mackowiak et al. (1997), Blatteis (2003). Consequences of pathogen challenges such as fever, anorexia and other acute phase responses have been viewed as part of an overall coping mechanism by hosts challenged by pathogens (Hart, 1988; Kyriazakis et al., 1998). Fever may be beneficial to the host as bacterial and viral growth rates are sensitive to changes in their ambient temperature, or, through the increased body temperature having positive effect on the host immune responses. Jiang et al. (2000) demonstrated that the latter of these two routes was the most significant, and Blatteis (2003) have reviewed further evidence in favour of fever having a beneficial effect on host immune responses during exposure to certain kinds of pathogens.

It is, therefore, important to try and relate the ability of an animal to mount fever to the effectiveness of its immune response. Furthermore, Bradley and Kauffman (1998) found that protein malnutrition may reduce the extent of fever. This provides a further link between the immune response, which amongst other things depends on resources, and fever. Future experiments are warranted for investigating any interactions between protein partitioning, fever and the ability of an animal to cope with infection. This would be crucial for prediction by models that attempt to incorporate the effects of the physical (i.e. thermal) environment alongside those of the infectious environment on performance.

Existing rules of protein partitioning during disease were found to be qualitative, and testing their qualitative predictions against literature data showed that there is a competition for amino acids between growth and immune functions. This is contrary to the prevailing view that immune responses are prioritised over other body functions, when food resources are scarce (Klasing et al., 1987; Schrama et al., 1997; Lochmiller and Deerenberg, 2001). This competition for resources (and its fitness benefits) raises some interesting questions in terms of how an animal has evolved to cope with infection (Sheldon and Verhulst, 1996; Rauw et al., 1998). Pathogen challenges clearly have a significant fitness cost on a host as it can lead to mortality or severe morbidity (Rigby et al., 2002). In some cases, however, the consequences of a pathogen challenge may be rather small (Coop et al., 1985). This could have lead to animals being able to mount an immune response and partition resources for this that is proportional to the extent of a challenge, including pathogen virulence. It is likely that natural selection in nature would have occurred during high levels of infection and when resources were scarce. A more resistant genotype is one more able to deal with a pathogen challenge with lower pathogen loads and a lesser effect on food intake (Sandberg et al., 2006).

Attempts have been made to select animals that cope better with disease by different approaches in pigs (Wilkie and Mallard, 1998), chickens (Siegel and Gross, 1980) and sheep (Bisset et al., 1996; Douch et al., 1996) amongst others. In relation to the framework discussed here the important issues include the relationship between degree of resistance and (i) reductions in food intake, (ii) changes to requirements for mounting an immune response and (iii) the extent of partitioning towards either growth or immune functions by the host. Different methods have been used to attempt to select for more resistant genotypes. Wilkie and Mallard (1998) selected for high and low responder pigs in terms of general immunity by using an index for several immune traits when challenged by several different types of antigen. Siegel and Gross (1980) and Yunis et al. (2000) selected chickens for a single immune trait (either high or low antibody responses to sheep red blood cells). Genetic selection in sheep against gastro-intestinal parasitism uses a proxy measure of the level of challenge (faecal

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egg counts), which has been found to have a strong relationship with reduced worm burdens (Bisset *et al.*, 1996). Unfortunately, there is an absence of evidence of controlled challenge trials of strains of different resistance, where food intake, performance and measures of immunity have been recorded. This may have allowed for the consequences of genetic selection for resistance to be better understood.

Wilkie and Mallard (1998) selected for responses in both cellular and humoral immune components to a range of antigens in attempt to achieve *general* resistance (Magnusson *et al.*, 1997, 1998 and 1999; Reddy *et al.*, 2000). Magnusson *et al.* (1997) showed that pigs selected for high immune responses were more responsive to vaccination (indicating a greater ability to recognise antigens) and *LPS* challenge. Antibody responses were greater, and the maximum antibody level occurred sooner than in pigs selected for low immune responses. This would suggest that more resistant animals would have a higher requirement for immune proteins at lower pathogen loads. The high response lines, however, achieved 90 kg over a shorter time than the low response lines (Wilkie and Mallard, 1998) when challenged by other pathogens. This may suggest that while more resistant genotypes invest more resources in immune responses, there is also a net benefit on growth. Gross *et al.* (2002) also found that chickens selected for resistance as by the method of Siegel and Gross (1980) had less damage when challenged with *Mycoplasma gallisepticum*.

Selection for high immune responses may not always be beneficial. In a challenge experiment with *Mycoplasma hyorhinis* in pigs, the high response lines had a greater incidence of arthritis (Magnusson *et al.*, 1998), attributed to higher activation of the immune response. An inevitable consequence of immune system activation is a non-specific immunopathology that does not confer any obvious advantage to the host, except for the overall positive outcome of a challenge. An improved general understanding of resistance and its effects on growth and performance is needed as different pathogens are dealt with by immune responses with different relative influences of innate, cellular and humoral immune responses and higher immune responses may not always necessarily lead (or indicate) to improved resistance (Adamo, 2004). There is some weak evidence in the literature, which suggests that animals selected for faster growth were affected to a greater extent when challenged by pathogens (Praharaj *et al.*, 1995; Yunis *et al.*, 2000; Huff *et al.*, 2005). This has been attributed to the prioritisation of resources towards growth rather than immune functions (Rauw *et al.*, 1998). It is essential, therefore, for future experiments to consider well defined genotypes (differing both in terms of genetic growth potential and resistance) when challenged by pathogens.

In future work we will present some possible quantitative solutions to the problems that have been raised in this review. It will be necessary to have an adequate scaling rule for describing pathogen challenges, which accounts for how animals of different sizes and degrees of maturity respond to a pathogen challenge. Suitable functional forms need to be chosen that relate the level of a pathogen challenge to amino acid and energy partitioning and requirements. The effects of host resistance on the values of the parameters of these functional forms would need to be known to be able to predict the performance of hosts with different levels of resistance. The model could then be used to assess how different genotypes (both growth potential and degree of resistance) cope during pathogen challenges when given access to different kinds of food. The model, and its future parameterisation and testing through relevant experiments may improve our understanding of the relationship between host performance, and pathogen exposure in different environments.

Acknowledgements

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